## AD-A284 837

Contract No.

90MM0504

TITLE:

MicroSAINT model of fatigue assessment

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REPORT DATE 29 May 1993

TYPE OF REPORT Final Report

## PREPARED FOR:

U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012

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MicroSAINT Model of Fatigue Assessment

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Samuel G. Schiflett Johathan French

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There are more opportunities for fatigue related accidents when long or unusual duty cycles must be maintained. A means to plan for the likelihood of fatigue is described in this report. An equation was developed from results obtained in a 30-hour sleep deprivation study. These data were mathematically modelled and incorporated into the equation which also considers circadian variation in performance. A Micro-SAINT model of a complex human task, the commit action of a weapons director aboard an AWAC aircraft, was developed to estimate the consequences of fatigue. Strong linear trends existed in the data so linear regression techniques were used. Significant amounts of the variance were accounted for by the equation for both accuracy and response time variables. A 36-hour sleep deprivation study was conducted to verify the model. The predicted performance trough was earlier but about the same magnitude as that observed. The approach outlined here seems reasonable for designing an equation to incorporate fatigue into computer models of complex behavior. Refinement of the model is needed using longer sleep deprivation periods that extend farther into the circadian cycle. Curvilinear data modelling techniques also are needed to account for more of the circadian rhythmicity.

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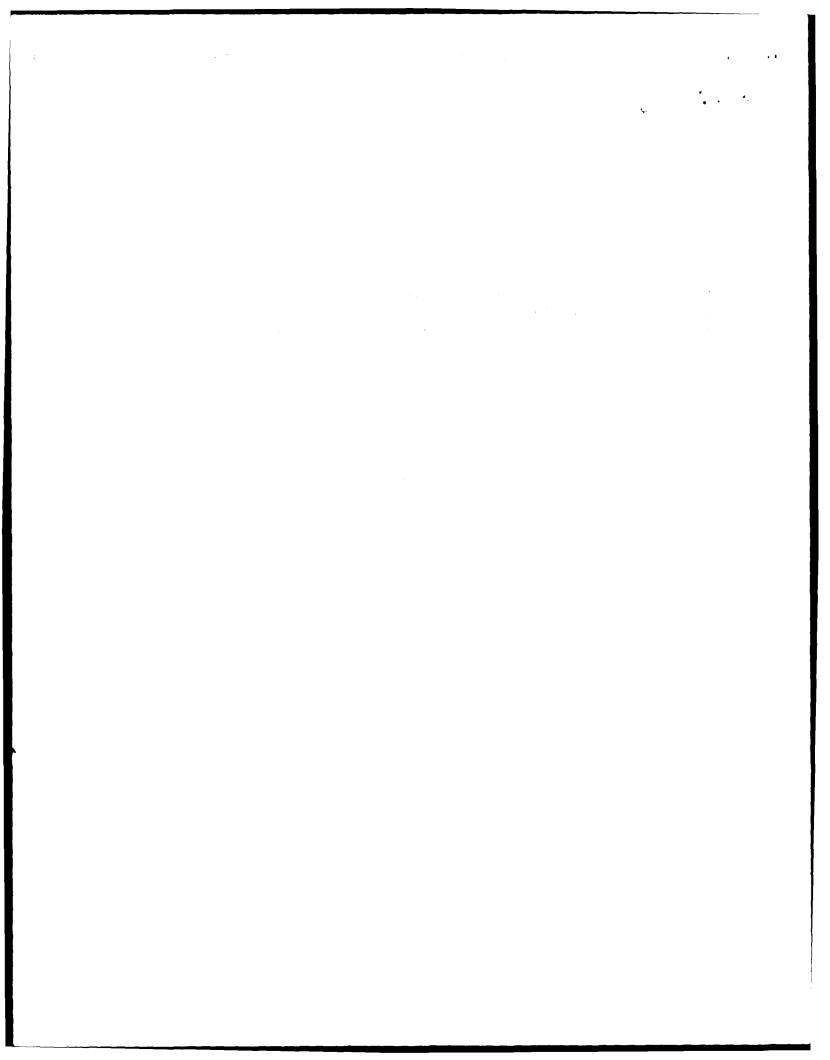
68

Cognitive, fatigue, models, MicroSAINT, sleep deprivation, computer models.

Unclassified

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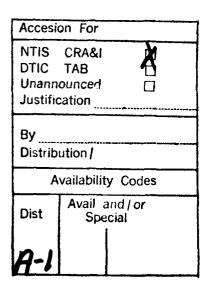
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Principal Investigator's Signature (Date

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## Micro SAINT Model of Fatigue Assessment FINAL REPORT 1993 (MIPR 90MM0504)

## INTRODUCTION

Understanding the effects of fatigue on performance is of critical importance to a society that places increasing reliance on operating services 24 hours a day. Shift work and compressed work week schedules are finding increased application in industry. Correspondingly, there are more opportunities for fatigue related accidents when long or unusual duty cycles must be maintained. Fatigue induced accidents have already produced dramatic catastrophes in nuclear power and chemical processing plants (US Congress OTA, 1991). The military has always depended on 24 hour operations and the complexity and potential danger associated with such equipment underscores the need for a thorough understanding of the effects and timing of fatique on performance. An awareness of the effects of fatigue on human performance may facilitate the development of counter-measures whether that be in the design of complex machinery or in personnel management. A means to plan for the influence of fatigue on performance would be useful to best utilize human resources. The purpose of this report is to describe the development of a mathematical model that is designed to predict performance degradation associated with sustained performance. Finally, a computer simulation of a complex cognitive performance is described which makes use of the model.

As better modeling tools have become available, models of human performance in a variety of environments and conditions have increased in number. Systems analytical models like MicroSAINT are used to develop computer simulations of human performance in clearly defined situations with varying degrees of workload and complexity. These models are restricted in their usefulness since they do not consider operator fatigue in their performance In fact, most models are idealized simulations that simulations. seldom make mistakes and are unaffected by ordinary human foibles like lapses in attention. Realistic models need to take into account human performance impediments to accurately estimate human performance. The focus of the research effort was to find a way to quantify impediments such as those which occur with fatigue induced by sleep deprivation and incorporate them into a model of performance.

A MicroSAINT computer simulation of tasks performed by a weapons director on an AWAC aircraft was selected as the model to be degraded by the fatigue equation. The task involves a complex and highly organized behavior that is affected by prolonged duty and circadian dysrhythmia. However, the fatigue equation was designed to be parsimonious and applicable outside of computer simulations as well. For example, managers could estimate the percentage decay in performance knowing when the circadian

performance trough occurs and staff the most affected shifts accordingly.

This effort was an attempt to predict cognitive performance decrements that are anchored in hours of sleep deprived performance. A distinct difference in the model from other estimates of performance is that it takes into account the circadian variation in performance due to human fatigue. Thus, the model is a function of hours awake as well as circadian rhythmicity. The predictions of the model need to be tested and refined further, efforts which were in progress when the contract period ended. It is hoped that the general direction of the model development and its application as described in this paper will provide guidance for future such efforts.

## METHODS AND RESULTS

The first of 2 experiments was conducted to evaluate the effects of prolonged duty (30 hours) on cognitive performance The study also concerned performance under bright illumination in a repeated measures, counter-balanced bright light (3000 lux) compared to dim light (100 lux) illumination. Only data collected from the dim light sessions were used in the modeling Data for the bright light results, which effort described here. was an important consequence of this contract (Appendix 5), can be found in other sources (French; 1990, 1991). During the study, 9 male subjects were required to perform a 45 minute standard triservice performance assessment battery every 120 minutes. Subjects were non-smoking civilian and military personnel and were required to refrain from all drugs for 24 hours and to get a normal evening rest prior to testing. The computer tests were developed by the Office of Military Performance Assessment Technology (OMPAT) and were based on established cognitive tests (Hegge, 1985). Subjects received training trials on the computer battery over a 10 hour period, beginning at 1000. At 1800 hours, the study trials began and lasted until 1000 the following day. In addition, EEG, EOG, blood samples and oral temperatures were taken. Primary interest concerned the cognitive skills degraded by fatigue as measured by the performance battery.

Five subjects were tested at a time and each subject was assigned to an individual testing booth that contains a wide spectrum fluorescent illumination source and a PC workstation. Each booth was separated from adjacent booths by sound attenuating frame partitions that restricted the subject's view to their workstation. A comfortable chair allowed the subject to sit close to the workstation. The light source fixture is suspended on a wooden frame over each workstation and was adjusted to administer either the bright or dim light treatment. All of the subjects signed a consent form to participate and all were interviewed to determine that none suffered from sleep disorders. A sublingual

temperature reading was taken immediately following each cognitive test battery sequence. The subjects were required to stay in their testing booths throughout the study with the exception of short (< 5 minute) restroom breaks. Social interactions between subjects were kept to a minimum by the experimenter and by the demands of the testing schedule. There were at least 2 weeks between the dim and bright light experimental sessions for each group to allow reentrainment of the circadian cycle and recovery from sleep loss. The same foods were served to the subjects in their booths during both light conditions and at the same times.

Performance tests sensitive to fatigue were then determined by using paired t-tests to compare baseline performance (an average score obtained between the 1800 and 2000 hour performance trials) to every 2 hour performance block. Tests that showed a significant difference from baseline at any of the 120 minute time points were used. The variables from these tests that were significant were categorized as accuracy or response time variables. Finally, the scores were normalized (percent change from baseline score) and included in a composite accuracy or response time scores.

Many attempts were made to mathematically define the performance curves that were generated. Multivariate equations were generated that included eye blink and oral temperature but these curves did not improve on a the predictability of a linear decay There were strong linear trends in the data, in fact linear regression analysis described the data better than other multivariate equations. In addition, the model lacked a variable that could account for circadian dysrhythmia, such as that which occurs during atypical or extended work periods. Accordingly, the neuroendocrinological data became available and melatonin values were considered as a means to permit the equation to have a circadian sensitivity. Figure 1 shows the normal melatonin values that were generated in the fatigue experiment for the dim light subjects and melatonin suppression by bright lights. follows a circadian pattern that parallels the human sleep wake cycle. In fact, ingestion of melatonin is reported to induce fatigue in rested individuals (Lieberman, et al 1984). Adding melatonin values significantly improved the performance model over simple linear equations. Accordingly, melatonin values were fitted to the equation for composite accuracy (Eq1) and composite response time (Eq2) as shown in Table 1.

Estimates of the variance accounted for  $(R^2)$  for the linear description of the composite accuracy scores was 12.4 % whereas with the inclusion of the melatonin weighting this value increased to 19.9%. These results are shown graphically in Figure 2. Figure 2 shows the equation (Eq1) estimate which includes the melatonin weighting fitted to the composite accuracy score. As well, the  $R^2$  for composite response time variability was increased from 36.7% considering only the linear component to 36.9% with the melatonin weighting. These results are shown graphically in Figure 3.

TABLE 1. Accuracy (Eq1) and Response time (Eq2) prediction equations. Accuracy Performance Score = Y + H(HA) + M(CV)(Eq1) Where Y = Y intercept (148.3); H = Hours Awake weight (-2.69107);HA = Hours Awake (range between 12 - 30 hours); M = Melatonin weight (-0.54794);CV = Circadian value (values from Figure 3). Response Time Performance Score = Y + H(HA) + M(CV)Y = Y intercept (81.6);H = Hours Awake weight (1.23);HA = Hours Awake (range between 12 - 30 hours); M = Melatonin weight (0.0139);CV = Circadian value (values from Figure 3).

The percent of variance described indicate that accuracy predictions were better predicted by the circadian component (12.4% vs 19.9%) than was response time (36.7% vs (36.9%). In each case however, a significant (p<.001) proportion of the variability was associated with the performance predictions. Use of the melatonin data had the additional advantage of incorporating a circadian component to the equations and served to decelerating the linearly accelerating estimate line as the melatonin values waned. Appendix 1 shows the changes in R<sup>2</sup> for accuracy and response time variables considering all time points and then excluding later time points. Overall, R2 is greater as hours awake is increased. Correspondingly, less variance is accounted for during the early time points, presumably when subject variability would be greatest. comparing Figures 1 with Figures 2 and 3, it can be seen that the slope of the performance degradation is greatest between 14 - 20 hours awake which corresponds to the normal nocturnal surge in plasma melatonin levels shown in Figure 1.

Table 2 shows an application of the equation for composite accuracy estimations for 2 times, 1800 and 2400 midnight. Thus, knowing the hours a subject has spent awake and whether the time of interest represents the subject's subjective day or night, performance can be estimated by the equations.

A complex cognitive performance task was then selected for modelling by MicroSAINT. Simple models of performance like those shown in Figure 4 for the serial math test can be degraded by

TABLE 2. Using the equation for composite accuracy (Eq1) the values used to generate predictions of performance for 1800 and 2400 midnight for an average subject.

Predicted	Actual	Υ	Hours Awake	_	Melato	onin
Score	Score	Intercept	Slope			Value
113.45	100	148.3	-2.7	12	-0.6	4.6
77.29	75.74	148.3	-2.7	18	-0.6	41.14

including the equations at key nodes in the network. For example response time might be degraded by applying the equation to the time required for completion of the 'Enter Answer' node in Figure 4. Because of the experience available onsite in AWAC simulation, a model of target selection by a Weapons Director (WD) was modelled in MicroSAINT. A depiction of the model is shown in Figure 5.

The model simulates all the steps taken by a WD from identifying an unknown aircraft as friend or foe to committing aircraft resources if necessary. It is much more complex than can be easily shown here. The cognitive processes reported to underlie the tests deemed to be sensitive to fatigue from the original fatigue study were obtained from the literature. The location of WD actions that would be most affected by these cognitive processes were identified in the simulation by a WD expert and the response time equation was used in the model to slow down the simulated responses. Of the 102 task nodes involved in the WD simulation, 34 were viewed as impacted by fatigue and degraded by the equation. The mean time for each node to complete was replaced with the equation based on The result of 20 applications of the MicroSAINT total task time. WD simulation at each timepoint is shown in Figure 6 along with the corresponding SEM. For example, after 28 hours awake the responses of the WD slowed from 39% to 43 %. The graph might be visualized as the average result of the performance of 20 WD's in dealing with targets.

In an attempt to verify the predictions of the model, a second fatigue study was conducted which lasted 36 hours, extending the sleep deprivation by 6 hours over the previous study. The study consisted of 9 male subjects between the ages of 21 and 32 years. As before, subjects arrived in the laboratory at 0600 and received training every 2 hours on the 2 hour computer test battery from 1000 until 1800, when the test began. The test continued from 1800 until 1700 the next day. All conditions and methods were

otherwise the same as in the 30 hour sleep deprivation study. Additional trials were obtained at 30 and 36 hours. Operation Desert Storm interrupted this study halfway through.

Saliva samples were taken at each 2 hr interval for melatonin assays. For comparison, two plasma samples were taken at the time points associated with the nocturnal pulse of melatonin for comparison with the salivary samples as shown in Figure 7. This figure demonstrates that saliva may be used as a non-invasive measure of plasma melatonin levels (see Appendix 5 and 6). was taken from subjects in the fatigue experiment at 2 times when melatonin values were expected to be elevated. Saliva samples were taken throughout the entire study for comparison. The melatonin surge can be seen in the saliva in Figure 7 and salivary levels compare favorably with the plasma levels, perhaps delayed by about an hour in the saliva as melatonin extravasates from plasma into the saliva fluid compartment. For the purposes of the equations however, melatonin values indicate when the subjective night occurs and when fatique effects would be at their greatest. Thus, if an individual awoke at midnight, during the subjective night, they are more apt to show a greater fatigue a few hours later than if they awoke at 0600, during the subjective day. The equation may account for these day night differences in the estimations by decreasing then increasing degradation respectively, as shown in Figures 2 and 3.

The fatique equation for accuracy derived from Fatique study I were fitted to the composite accuracy value obtained during Fatigue study II. These results are shown in Figure 8. The longer sleep deprivation times associated with the Fatigue II study show the circadian performance effects better than the Fatigue I study. Except for the rapid deterioration in performance associated with the prediction curve from Fatigue I, the performance trough is about where it was estimated to be. The better training associated with Fatigue study II is evidenced in the lower SEMs when compared to Figure 2. By 28 hours after the composite data for accuracy are similar to those estimated and then begin to recover by 30 and 36 hours of sleep deprivation. The equation for response time from The same findings for accuracy Fatigue I is shown in Figure 9. estimates can be applied to the response time estimates, although response time may be better estimated by curvilinear equations due to the circadian effects in Figure 9.

Tables 3 and 4 summarize the tests used that were sensitive to fatigue in Study II and the time of day where significant effects were found. Table 3 shows where performance was degraded, the circadian trough, and Table 4 shows where performance was improved. Graphs of significant performance effects from this study are included in Appendix 8. Rather than being sine wave components, the many graphs showing circadian effects show that the effects is more like a spike in the data. Data from Fatigue study I did not obtain enough sleep deprived performance to see the circadian rise

Table 3.

Significant Findings over 30-hr periods FATIGUE II STUDY

Eleven Cognitive Tests from the Complex Cognitive Assessment Battery & the Walter Read Performance Assessment Battery
 Multiple performance measures obtained from each
 Nine subjects (one group of 5; one group 0f 4)

Dim light sessions only

Streen trials per subject, 1 every 2 hours:

Performance decrements: Times at which performance is significantly worse (p < .05) than the baseline performance
(baseline performance is the average of performance in trials 1 & 2 (1000 and 1200 hrs));</li>

								TIME OF DAY	F DAY						
Measures	1400	985	1800	2000	2200	2400	0200	0400	0090	0800	1000	1200	1400	1600	Sensitive Tests
Nimber of trials															
Number of errors	2								2	-			-		manucol add & subjest meth, numbers, word 10
Mean RT								-	2	2		-	-		pen recogniston,choice RT, serial meth
RT of Correct								2		4		က	-	-	manitan pan recondos RT.col add & aub.word ID
Responses											-				pen recognidon.
Minimum RI		[-	-	-		<u> </u>		-	-		-	-	2	1	wilk,pen recog, choice RT
Task Duration							-	-	2	4	-		-		ombra, wilk log rang, pem ree. ono RT, senei math, stanione
Stanford SS Score							-	-	-	-	-	-	-		stantord eleopiness eurey
TOTAL	2	-	-	-	0	-	2	5	7	11	3	2	9	2	
] [ ]	,														

Table 4.

## Significant Findings over 30-hr periods FATIGUE II STUDY

Eleven Cognitive Tests from the Complex Cognitive Assessment Battery & the Watter Reed Performance Assessment Battery
 Multiple performance measures obtained from each

. Nine subjects (one group of 5; one group 0f 4)

. Dim light sessions only

Sixteen trials per subject, 1 every 2 hours:

II. Performance increments: Times at which performance is significantly better (p<.এ5) than the baseline performance (baseline performance is the average of performance in trials 1 & 2 (1000 and 1200 hrs)):

								TIME OF DAY	T DAY						
Moseuroe	1400	981	1800	2000	2200	2400	020	0400	0090	0080	1000	1200	1400	1600	Sensitive Tests
Measure of trials			2	2	6	2									mani.log rang, cot add & sub
Number of errors	2					-				2		-	2	-	n ping,mani,log rang, ool add 8. sub, pen racog, numbers
Moon RT			2		2	က	2								dus & bbs loo, par gol, mem
RT of Correct			-	2	2	6	2								maniten.log rang,serial math. ool add & sub
Responses Minimum RT	-	8	2	_		-									wildneon_manifen.log rang. col acid & sub.serial meth
					,										manikan, column add & sub
Maximum RT Task Duration	4	-			,		2								maniten,col add & sub, numbers, rt planning
Stanford SS Score															
TOTAL	_	4	7	5	6	5	9	0	0	2	0	-	2	-	

evident in performance found in Fatigue study II. Accurate models of performance beyond 28 hours of sleep deprivation need to include these longer sleep deprivation data.

## CONCLUSIONS

This project represents an attempt to mathematically describe the effects of fatique on performance. It may be the first to address the circadian sensitivity of performance prediction. only requirements for the equation are knowing how long an individual has been awake and whether it is the subjective day or Refinements to the equation need to increase the power of the estimate by adding more data to the normalized prediction line and to use longer sleep deprivation studies. Predicting performance should be validated by first estimating performance decay using the procedures described in this report and then empirically assessing it. The results from the 30 hours of sleep deprivation study should be combined with the results from the 36 hour study to get composite scores for accuracy and response time variables. As the contract ended, the authors were in the process of completing this and experimenting with curvilinear analysis for the prediction equation. Future work in this area needs to account for the marked performance spikes that occur in the circadian times.

A range of scores can be predicted for each hour on duty that could outline optimum levels of performance. This information would be useful to predict when human efforts are critically degraded and steps taken to keep performance above minimum. utility of the equation allows it to be used in systems analytical workstation models and to determine the impact of fatigue on these The WD model is an example of how this might be accomplished for other MicroSAINT or similar programs. Finally, results might be used to recommend when additional operators or relief crews might best be employed once individual performance falls below a critical ability. The work requires careful evaluation of MicroSAINT models for the appropriate nodes to add the equation generated by the equation presented here or those from other fatigue studies. Longer fatigue studies should show greater weights to the hours awake value and even greater weights from the The results may extend to non-computer circadian variable. applications as well in planning for the effects of fatigue on shift workers or preparing for acute sustained operations.

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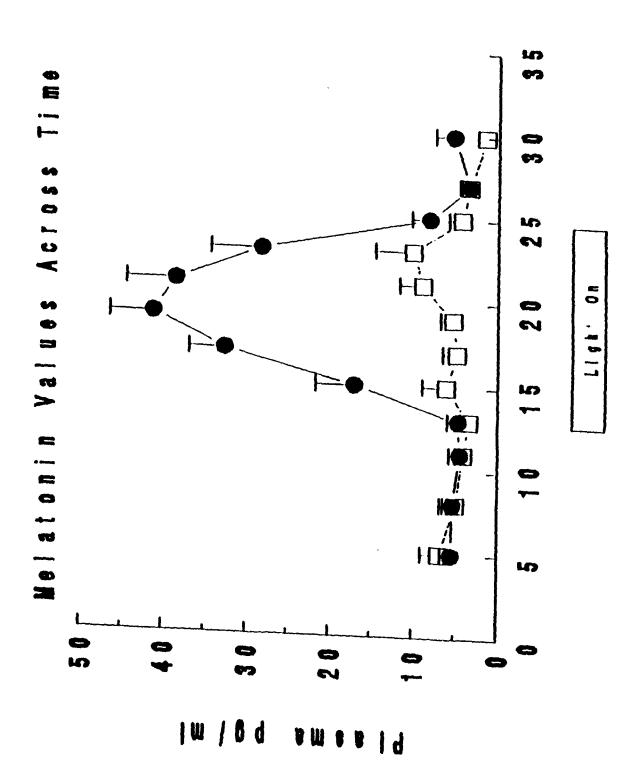
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Lieberman, H.R., Waldhauser, F., Garfield, G., Lynch, H.J. and Wurtman, R.J. Effects of melatonin on human mood and performance. Brain Research, 323, 201-207, 1984.

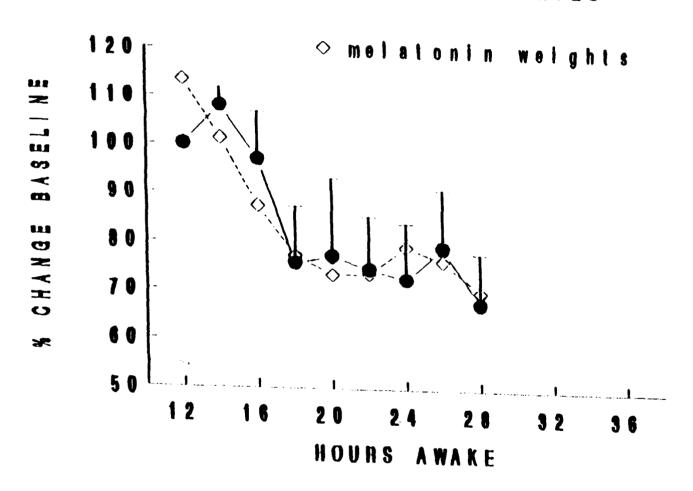
US Congress Office of Technology Assessment (OTA) <u>Biological</u> <u>Rhythms: Implications for the Worker</u>. (OTA-BA-463). Washington DC. US Government Printing Office, September 1991.

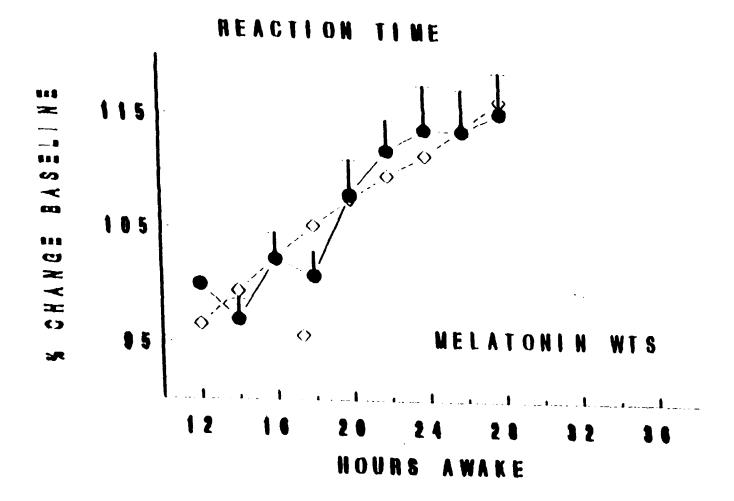
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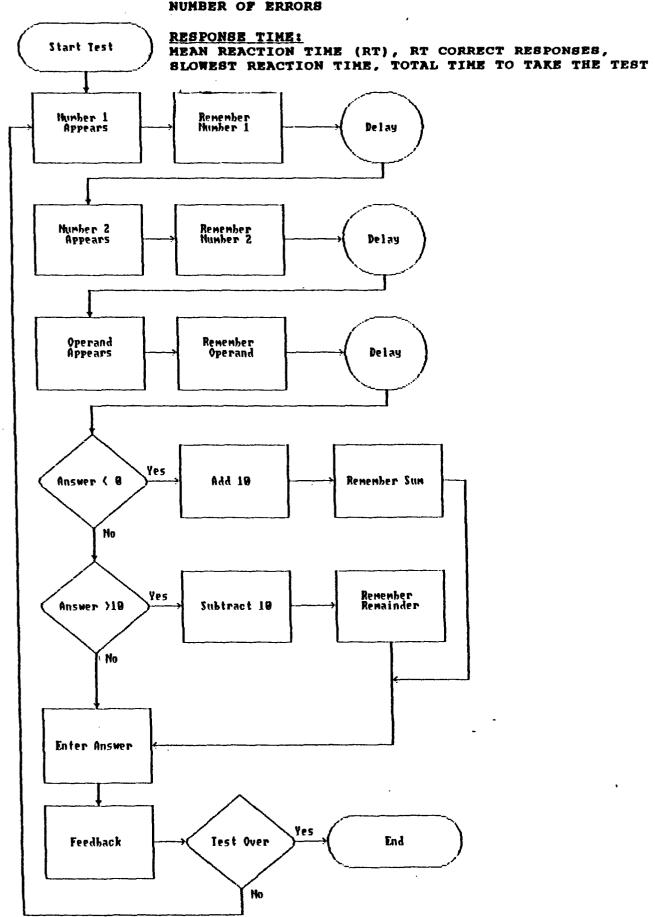
## ACCURACY VALUE ESTIMATES





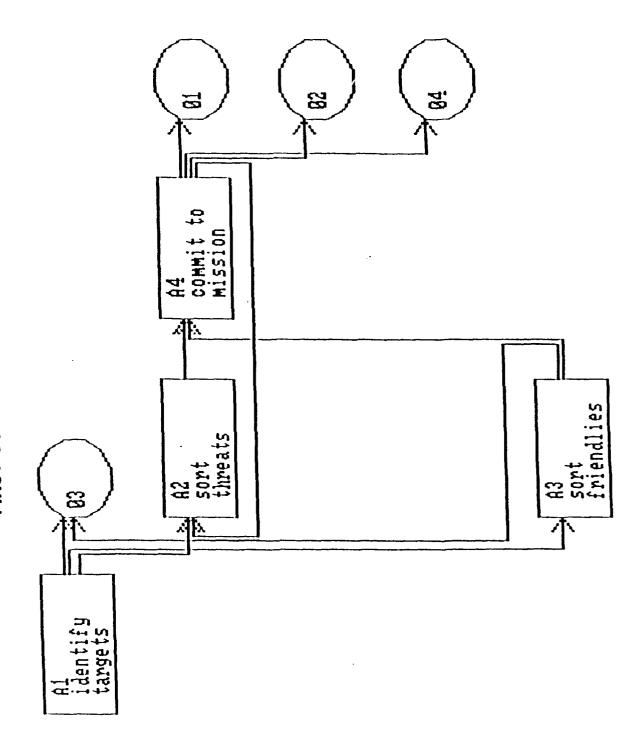
## **SERIAL ADDITION-SUBTRACTION TEST: UTC-PAB**

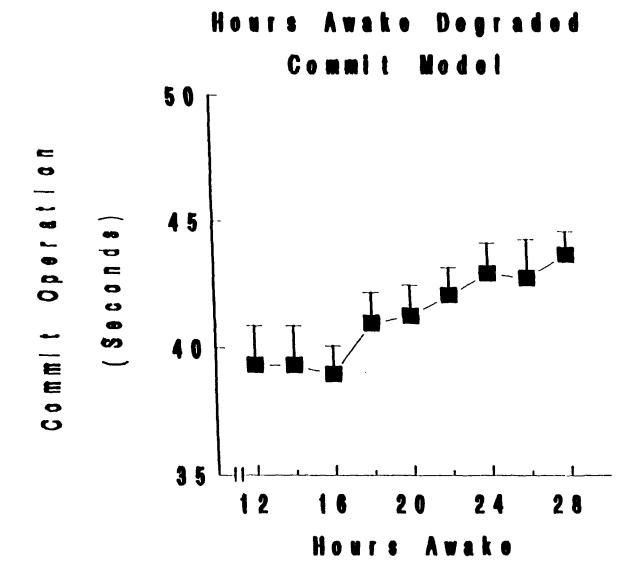
## ACCURACY: NUMBER OF ERRORS



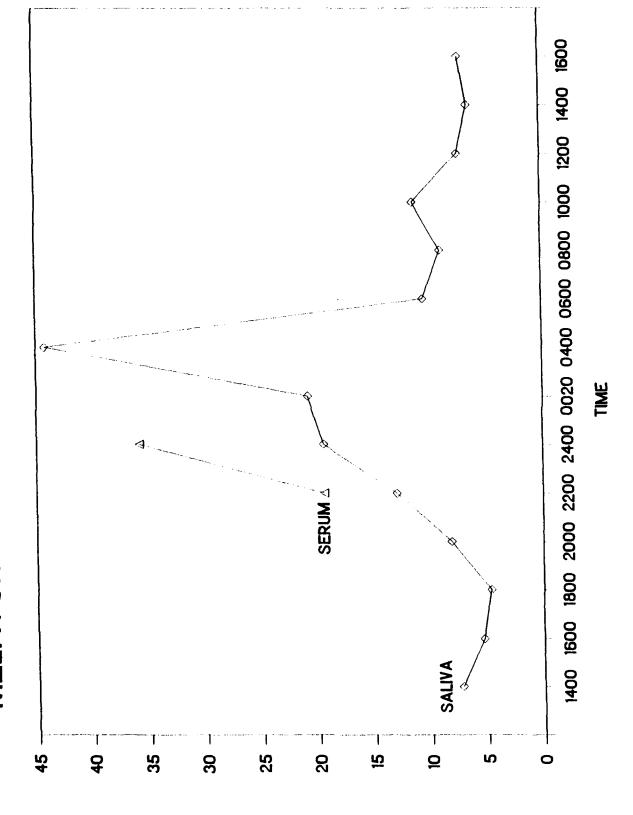
# COMPUTER MODEL OF WEAPONS DIRECTION (AWAC) PERFORMANCE

## FIRST COMPLEXITY LEVEL



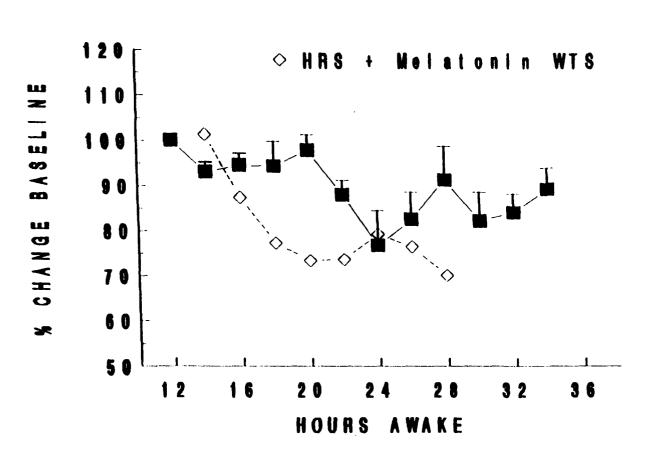


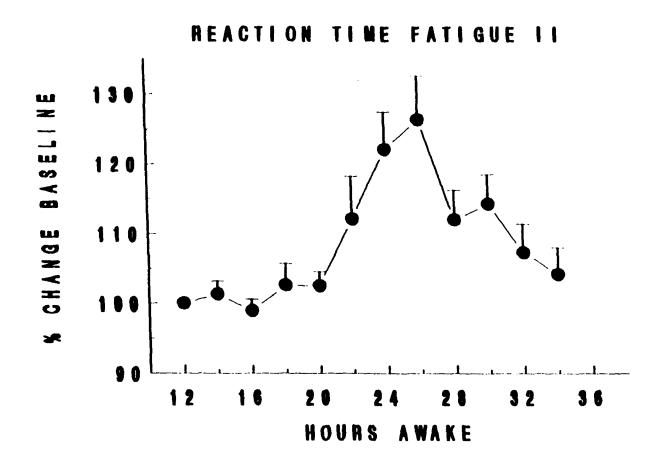
## MELATONIN LEVELS IN SERUM AND SALIVA



PG/ML

## ACCURACY VALUES FATIGUE II





## PROGRAM OVERVIEW

Use Tri-Service performance battery; mo∩d; physical strength; psychomotor; EEG, EOG; neuroendocrine levels; temperature

Evaluate Micro-SAINT fatigue model on complex behavior and incorporate model into larger simulations of behavior



Develop 30-hour awake fatigue model and make test predictions for 36-hour fatigue using Micro-SAINT generated model of performance

Determine which measures are most sensitive to fatigue and recommend field assays to monitor fatigue

Develop generalized model of human performance which can be used to accurately predict the impact of fatigue or military performance expectations

APPENDICES

Appendix 1 Reduction in R<sup>2</sup> considering various times in the fatigue estimates

The  $R^2$  values for the linear Hours awake (HA) and the circadian sensitive curvilinear equations HA + Melatonin weighting (MW) for select time points in the fatigue study and the associated probability values (p). The variance accounted for values includes the data up to each Hours Awake indicated.

Hours Awake	Accuracy	p Respon	ıse	Time %	p
28 Hr					
на	12.35	0.003	36.	. 7	0.0001
HA+MW	19.94	0.0002	36.	.96	0.0001
26 Hr					
HA	10.72	0.005	35	. 62	0.0001
HA+MW	18.90	0.0007	35.	.74	0.0001
24 Hr					
HA	14.10	0.0024	34	. 93	0.0001
HA+MW	21.03	0.0008		.94	0.0001
22 Hr					
на	13.86	0.0056	30	. 58	0.0001
WM+AH	22.69	0.0014	30	. 68	0.0001
20 Hr					
на	12.61	0.0167	16	.36	0.006
HA+MW	24.38	0.0028		.73	0.0214
18 Hr					
на	13.93	0.025	2	.51	0.356
HA+MW	29.62	0.003	3	.53	0.553
16 Hr					
НА	0.46	0.738	3	.27	0.367
HA+MW	24.83	0.033	3	.37	0.663

## Appendix 2 Related Publications during the quarters

The prediction of cognitive performance degradation during sustained operations. Paper presented at the Proceedings of the Human Factors Society Annual Meeting, Oct. 1992, 9 pages.

Predicting fatigue effects on performance. French, J, Neville, K., Rowe, A., Eddy, D. and Schiflett, S. Proceedings of the Aerospace Medical Association, 74, 1992.

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## Appendix 3 Milestones during the contract

### 1990

PROGRESS: The first of 2 sleep deprivation studies obtained cognitive performance data from 9 individuals during 30 hours awake These data provided the basis for the Fatique (Fatique I). equation. The final report of data collected during Desert Shield was made to the MAC surgeon general's office. Fatigue data from Desert Shield provided useful insight into operational fatigue evaluation and the utility of fatigue models. The principle investigator. became chairperson for the HFE SusConOps TAG permitting greater contact with tri-service fatigue investigators. The physiological data from Fatigue I study was determined (melatonin, cortisol, temperature data). Microsaint animation program was purchased and implemented into models of cognitive fatique. Polyvariate equations of fatique (combining temperature, composite performance and hours awake variables) were determined for 30 hours sustained performance. Predictions from the equations were made for an upcoming 36 hours awake study.

## 1991

Interruption in the MicroSAINT model of fatigue effort PROGRESS: occurred again because of the Gulf war. During the final days of Operation Desert Storm, an improved operational study was designed to take advantage of the maximum flight hours and minimum crew rest experienced MAC air crews and data collection began. sensitive to fatigue were distinguished as reaction time or accuracy variables as a result of Fatique I study. The protocol for this second sustained fatigue study (Fatigue II) was approved. Data collection began for the Fatigue II study. circadian aspect of the melatonin data improved the predictability of the Adding this periodic variable to our equation fatigue equation. generated a linearly degraded oscillating performance curve, accelerated at certain times of day by the melatonin weighting and slowed at other times (diurnally) by the (nocturnally) Fatigue scores could then be obtained for melatonin weighting. accuracy and performance values by knowing only the number of hours awake and the time of day.

## 1992

PROGRESS: A MicroSAINT model of Commit switch action for Weapons Directors onboard an AWACS aircraft was developed. The equation for composite reaction time was used to degrade the completion times for the average Weapons director. Also during this time a salivary assay was developed for Melatonin which will greatly facilitate the assessment of this important circadian marker. Graphs and statistics were completed on Fatigue II. Work was progressing to refine the equation with longer sleep hours, more

subjects and better training and curvilinear analysis when the support period ended. Future efforts should focus on modeling the 36 hours and longer to better account for circadian variation and in validating the predictions in subsequent work. Composite Accuracy variables should be added to our Weapons director MicroSAINT simulation to further "humanize" the model.

## Appendix 4. List of personnel payed by contract support

The following people contributed efforts to the development, analysis or design of the model described in this report.

Dr Douglas Eddy, Ms Becky Cardenas of NTI Dayton OH assisted with every stage of the analysis and in the incorporation of the fatigue equation into the commit switch model (Figure 7).

Mr Neal Takomoto, Mr Phil Tessier and Mr Mathieu Dalrymple of SRL Dayton OH were responsible for the Weapons Director MicroSAINT model (Figures 5 and 6) and the actigraph analysis software (Appendix 7).

Dr George Brainard Thomas Jefferson University Philadelphia PA and Dr Russ Reiter Univ of Texas Health Science Center San Antonio Texas were consultants for neuroendocrine portions of this report (Figures 1 and 8; Appendix 6).

No funding was used for government personnel Drs J. French, S. Schiflett and Ms K. Neville.

## Appendix 5. List of Products as a result of this contract

- 1. The bright light study supported by the MIPR was the first to report human performance improved by bright illumination at night. These data were included in the Proceedings of the meeting of the Chronopharmacology Society in Nice France, in March of 1990, about 4 months before Dawson and Campbell published similar findings in Behavioral Biology. Other have since found further support for this phenomenon presumed to be mediated by bright light suppression of melatonin.
- 2. Dr Rus Reiter has developed a sensitive assay for salivary melatonin as well as plasma melatonin. The salivary samples are much easier to take and well correlated with plasma levels. The assay is described in Appendix 6. Although others have demonstrated salivary assays for melatonin, Dr Reiters procedures are more routine while just as sensitive as more tedious assays. These data should encourage and facilitate other researchers to utilization of melatonin in their studies of circadian variation.
- 3. Actigraphs were purchased to ensure the subjects in the sleep deprivation studies were normal in their sleep patterns before and after the study. The contract was very valuable in helping apply techniques to analysis of airlift crews during an opportunistic study. The actigraphs for example, were used for a sustained operations study of aircrew during Operation Desert Shield and Desert Storm. Software to expedite the analysis of actigraph data, called "Actigraf" was also supported by the contract. The program easily converts data files from the actigraph to Spreadsheet ASCII compatible form and is included in the appendix 7. The software is available on diskette from J. French or S. Schiflett of AL/CFTO, 2504 D Drive STE 1, Brooks AFB, Tx 78235-5104 (210) 536-3464.
- 4. ILS software for Quantitative EEG analysis were implemented and have received limited use. Much more use is expected to be made of the software in the near future.
- 5. This report can be considered a contribution to further work in the area. It represents the first effort to predict fatigue in human cognitive performance that is sensitive to circadian variation. The effort was progressing into the area of validating and enhancing the mathematical models developed and the MicroSAINT model of Weapons Director activity. The third year effort focused on using more subjects, longer sleep deprivation periods and curvilinear analytical techniques when the support period ended.

Appendix 6. Salivary assay for melatonin and cortisol

## The Guildhay Radioimmunoassay - A Direct Assay For Melatonin

In Dr. Reiters' lab, we employ the direct assay for melatonin as validated by Webley et al<sup>41</sup>. But, instead of using the double antibody technique for separation, we employ separation with coated-charcoal<sup>38,39,45</sup>.

## Sample Collection:

Dr. Reiter summarizes: "To adequately study the circadian production of melatonin it is necessary to collect pineal, blood, or other fluid samples over a twenty-four hour period. Since the bulk of the melatonin is produced at night, the most critical samples are those collected during darkness. For all species, including humans, it is recommended that samples (tissue or fluid) be collected at four hour intervals during the day; at night this interval should be reduced to two or three hours. In a few species, e.g., the syrian hamster, domestic mouse, and gerbal, the melatonin peak may be of very short duration. Thus, for these species it may be necessary to collect samples hourley at certain portions of the night. If the purpose of a particular study is to investigate some aspect of melatonin, regaurding its four hour rhythem, it will be necessary to vary the tissue or fluid collection times accordingly."

## Sample Preparation:

## For Pineal Gland:

After collection, each pineal is deposited in a frozen microfuge tube and placed on dry ice. When all pineals have been collected, bags are labelled and placed in a -70°C freezer, until the appropriate time to run the assay. The pineals for mice and rats are sonicated whole with 100µl of 0.05M sodium phosphate buffer (pH 6.8) PBS. Depending on your expected melatonin levels, 10µl of sonicate may be taken and saved in a microfuge tube for the melatonin assay, as well as other aliquots for other assays. If you are not scheduled to run melatonin that day, immediately freeze the microfuge tube on dry ice, and then transfer to -70°C freezer until assay. On day of assay, add 1020µl of Tricine buffer to each sample tube, vortex and dispense 250µl in duplicate for assay.

## For Serum:

Whole blood is collected into lithium heparin tubes\*§ and centrifuged, or let samples set to clot at 4°C. Centrifuge at 2000 rpm for 15 minutes. Then, 1000µ1 aliquots of serum are placed in microfuge tubes and frozen on dry ice. Labelled bags are placed into -20°C freezer until time of assay. Serum samples are thawed overnight in 4°C refrigerator for day of assay use.

#### For Saliva:

After collection of about 2-3 mls, centrifuge at 2000g for 10 minutes to remove solids and store at -20°C in microfuge tubes until analysis<sup>44</sup>.

- \* The anticoagulants most commonly used in primate blood grouping practice are ACD (acid citrate dextrose) or EDTA (ethylenediaminetetraacitic acid)<sup>4</sup>.
- § Recent reports suggest that heparin can interfere with melatonin assays 42.

# Reagents:

A) Tricine Buffer

All water used is fresh, reagent grade, in glass.

For 1 liter with H<sub>2</sub>O:

17.9 gm Tricine (Sigma Chemical Co., or Aldrich Chemical Co.)

9.0 gm NaCl (ACS)

1.0 gm Sodium Azid, NaN<sub>3</sub> (Sigma Chemical Co.)\*

1.0 gm Gelatine (ACS)

Then, it is necessary to keep the solution continually stirring and heat to 50°C (or less) for 15 minutes to dissolve gelatin. Cool to room temperature. Then, adjust pH to 8.0 with 4N NaOH.

(Begin pH 5.5). Buffer is made up fresh, weekly.

\*Caution: Sodium Azid is Highly Toxic!

When dissolving gelatin, never let temperature reach above 50°C.

B) Antiserum

The antiserum is supplied by STOCK GRAND LTD (p.11), freeze-dried. Each vial is re-constituted with 2 ml double-distilled water to provide an intermediate dilution of 1:10. This is aliquot into 22/11 portions and stored at -20°C. The working solution is made as required for assay by diluting one 22/11 aliquot to 20 ml with Tricine buffer. This provides sufficient reagent for 200 tubes with an initial dilution of 1:9000.

## C) Label

The <sup>3</sup>H-Melatonin is obtained in quantities as stated on p.11.

A new batch of label should be stored at -20°C. An intermediate solution of <sup>3</sup>H-melatonin is prepared by diluting a 1mCi/ml stock solution at 1:100 with ethanol. This is stored at -20°C. The working solution is prepared by further diluting the intermediate solution with Tricine buffer to 2,000 cpm @ 100H per tube (1:220).

## D) Standards

- [1] 1mg melatonin (Sigma) to 1ml EtOH (200 proof; 1mg/ml),
- [2] 50µ1 of [1] to 950µ1 tricine buffer ( 50µg/ml ),
- [3] 40µl of [2] to 960µl tricine buffer ( 2µg/ml ),
- [4] 1ml of [3] to 99ml tricine buffer (20ng/ml)

Aliquots of [4] are stored at -20°C (125µ1/vial) = Stock.

RIA procedure: (In 13 x 100 mm tubes)

- 1) Thaw one vial of melatonin standard stock to do one duplicate set of standard curve tubes.
  - 2) Add 100µl stock to 1900µl tricine buffer, vortex, ( tube 1 = 1000pg/ml ),
- 3) Prepare 9 additional tubes, containing 1000/ll tricine buffer each ( tubes 2 thru 10 ),
  - 4) Transfer 1000µl from tube 1 to tube 2, vortex (500pg/ml),
- 5) Repeat step 4 for each following tube, i.e., take 1000/11 from tube 2 to tube 3, from 3, 1000/11 to tube 4, etc.. Standard Serial Dilution.,
- 6) At end, transfer 1000/11 from tube 10 to waste. Now, all tubes contain a total of 1000/11.

Take 250/11 from each tube, in duplicate, into 12 x 75 mm tubes to run in assay.

The concentrations of melatonin are:

Tube pair # 1		2	3	4	5	6	7	8	9	10
pg/ml	1000	500	250	125	62.5	31.25	15.6	7.8	3.9	1.95
pg/tube	250	125	62.5	31.25	15.6	7.8	3.9	1.95	0.97	0.48

### E) Dextran-Coated Charcoal

Suspend charcoal (p. 12) at .5% in tricine buffer, add .05% Dextran (p. 12). Make up fresh each day for use. Let stir continuously for 30 minutes at 4°C. Then, use immediately for the termination phase of the assay.

## F) Scintillation Fluid

This is stored in solid plastic containers. The solution is called Liquiscent from National Diagnostics. It is a standard solution for liquid scintillation counting.

## Radioimmunoassay Procedure

The assay is performed in 12 x 75 mm disposable glass tubes. All samples are done in duplicate. Sample or standard volumes of 250H are dispensed into the vials followed by 100µl antiserum (AS=Sheep Antibody to Melatonin p. 14) and 100µl [3H]melatonin (p. 14). Total (T) tubes contain only 850/11 tricine buffer plus, 100µl [3H]melatonin. Non-specific binding (NSB) tubes contain 350µl tricine buffer plus, 100m [3H]melatonin. Binding (B) tubes contain 250m tricine buffer, plus 100/11 antiserum, plus 100/11 [3H]melatonin. The vials are mixed by vortexing when all additions have been made, and placed in refrigerator (covered with foil) at 4°C to incubate overnight. The next morning, the dextran-coated charcoal solution (above) is made and set to stir continuously for 30 minutes at 4°C. Then, add 500<sup>11</sup> of charcoal solution to all tubes, except total tubes, vortex for 1 second, and begin the second incubation for exactly 15 minutes at 4°C (in refrigerator). Timing is began with the first tube addition of charcoal. It is very important to add the charcoal as quickly as possible to each tube, so use an Eppendorf Multipette<sup>R</sup> pipette. After incubation, tubes are immediately centrifuged for 15 minutes, 3000 rpm, at 4°C. Then, 750/11 of supernatant is removed, along with 750/11 from the total tubes. Be very careful not to disturb charcoal! Place the supernatant into clean, 20 ml, glass, scintillation vials. Add 7.5 ml scintillation fluid, shake well, and count vials for 10 minutes using the  $\beta$ -counter.

# Extraction Procedure (if applicable)

Extraction with organic solvents is often used in assays for small hapten molecules, such as the indoleamine and steroid hormones, in cases where the binder alone does not confer adequate specificity. Some type of solvent extraction is desirable for hormones which circulate in the picomolar range. Failure to employ an extraction procedure can lead to over-estimates of low levels and to non-specific effects due to materials such as fatty acids. Solvents must be of the highest possible purity and checked frequently by evaporating solvent alone and determining the "blank" value in the assay.

## Materials:

Chloroform ( ACS, glass distilled )\*
Dry Ice
Methanol ( ACS ) or Acetone ( ACS )\*\*

\* Toxic! Do Not Inhale.

\*\* Acetone is Corrosive!

Double Distilled Water Petroleum Ether (ACS)\* Tricine Buffer (p. 15)

### Procedure:

Set up standard curve and samples in single samplings of 60001 each in 12 x 75mm culture tubes. Set up 3 NSB and 3 B tubes with 600H of buffer. Totals (T) are not extracted. If you are working with less than 600H of sample, add enough buffer to bring the total volume to 600|1. Add 2 mls of chloroform to all tubes. Vortex well. Place tubes in a wire rack. Place racks in dry ice-bath ( dry ice and methanol or acetone) for 1 to 1.5 minutes, depending on the amount and spacing of the tubes, to freeze the organic and aqueous layers. Thaw racks about 30 to 40 minutes. Centrifuge tubes at 4°C, 3000 rpm, for 10 minutes. Aspirate only the top aqueous layer. Leave bubbles and white middle layer intact. Use slight vacuum. Then, add 500/11 double distilled water. Vortex well. Centrifuge tubes at 4°C, 3000 rpm, for 10 minutes. Aspirate aqueous layer, as well as the chloroform down to 1 ml. Evaporate this volume in a vacuum oven for 1.5 hours at 80°C, 25 psi, or leave in ventilated hood overnight. Let tubes cool to room temperature. Add 600/11 tricine buffer to all tubes, vortex well, and let set to elute overnight in refrigerator. Add 2 mls of petroleum ether to all tubes. Vortex well. Centrifuge tubes at 4°C, 3000 rpm, for 10 minutes. Aspirate carefully. Do not pull off bubbles! Then, let tubes set for 4-6 hours, or overnight, in hood so that any petroleum ether may evaporate. Then, take duplicate 250/11 aliquots from all sample and standard tubes into 12 x 75mm tubes. Take 350/11 from each NSB tube into one each 12 x 75mm tube. Take 250/11 from each B tube into one each 12 x 75mm tube. Prepare total tubes and perform the assay procedure as described above.

# **Computer Calculations**

Briefly,

we use Lotus 1-2-3. Everything on the worksheet has been pre-assigned and locked in. So, all that is necessary for you to do is, key in your counts (cpm). The function key F9 will calculate formula answers after you have provided the information. After plugging in your counts for the standard curve, press F9. [Remember, the amount of cold melatonin (as known standards, or unknown samples, respectively) varies between the tubes, so the amount of anti-serum bound hot melatonin is reciprocal to the present concentration of cold melatonin.] Then, perform the curve regression. The curve is in the log (mg/ml) logit (B/Bo) form. To start, begin at the menu (press "/"). Push "D" for data, then "R" for regression. Highlight x-range and y-range to make sure every column is correct. Highlight output range, which is the one space beside "Regression Output" on the screen.

Then, press "G" for go. After the regression is performed, push F9 to recalculate the slope and x-intercept. To view the curve, go to the menu ( "/" ), push "G" for graph, "V" for view. "Q" will quit the screen. You must save this graph if you want to print it later. Next, make sure the Regression Output is linear. The "r" value ( Correlation Coefficient ) must be 0.99 for a straight line. The "r²" ( Coefficient of Determination ) is the amount of varience contributed by the spread of data points from low to high values This can vary only by .01 from the "r" value. On our output, the slope is the "X-Coefficient", and the y-intercept is the "Constant".

Now, plug in your counts for each sample. Press F9. When working with serum of 250µl aliquots, the mean PG/ML calculation is your answer. When working with whole pineals, it is necessary to add an additional calculation  $t^{\mu}$  includes the Volume/Tube (V.T.) and Total Volume Of Homogenate (TV) used. Then we calculate PG/Pineal = (TV / V.T. \* Mean PG/ML) / 4.

# Some Rules Concerning The Assay

- 1) Never touch the surfaces of glassware or other equipment which might be in contact with the assay.
- 2) Whenever possible, use disposable glassware. Do not trust non-disposable glassware which had not been cleaned specifically for use in the melatonin assay. Do not use plastic disposable pasteur pipettes.
- 3) Never use other water than indicated here (p. 14), even if it is called "double-distilled". Check it. Never use water stored in plastic.
- 4) Do not attempt to do this assay in a laboratory where indoles ( especially melatonin ) are weighed out and used in µg to mg quantities.
- 5) Do not use shared reagents, even for people doing the same assay.
- 6) Be aware of the fact that several compounds, such as heparin, can interfere with melatonin assays. We recommend the use of lithium heparins, EDTA (p. 13).
- 7) It is very important that serum or plasma samples should not be haemolysed when assayed with this method. Haemolysis leads to a spurious increase in apparent melatonin, related to the degree of haemolysis, and due to quenching problems during counting.
- 8) Read and uncerstand each phase of the assay before you perform the assay.
- 9) The lab water is not used for melatonin buffer. We use the water from across the hallway (room 2.062V), which is a better reagent grade water.
- 10) Always dispose of the radioactivity properly.

Appendix 7. Actigraf: A program to summarize actigraph data

Systems Research Laboratories, Inc. Research & Development Computer Software Delivery Order 0009, Sequence 1, Attachment 2

We created a program **ACTIGRAF** to take downloaded wrist-unit data files and reformat them into standard text files. A listing of this program is included as Appendix E. This program contains several specialized options to meet the needs of the Desert Shield analysis effort. This program performs the following functions:

- Group raw wrist unit counts into buckets. The Desert Shield data was collected with both 2- and 3-minute counts. The bucket was used to create a standard 6-minute value. The program either calculates the bucket size based on the packing option, or accepts a bucket size from the user.
- Select a given day from the data. All other data is discarded.
- Apply a time zone correction. Some of the wrist units were programmed with Zulu time, while others were programmed with Eastern time. This correction adjusts all values to Zulu time. The program either calculates the time zone correction, or accepts a value from the user.
- Apply a count maximum. Some of the wrist units were set to record larger counts than others. This count maximum can be used to force a comparable set of values from wrist units programmed in different ways. The user must supply the maximum value, if desired.
- Output multiple data on a single line. The number of data to print on each line defaults to 1, but the user may specify another value. The program also outputs either header information, or data information, or both.

## Using the Progam

Type ACTIGRAF at the DOS prompt to display the following command line syntax and options:

```
Usage: F:\PHIL\BIN\ACTIGRAF.EXE [option...] <filename>
 Options:
   -ah
               - Auto Hour Set
   -b < bucket >
                   -- number of raw data to group
   -d<select day> -- Julian date to keep
   -h<offset>
                  - Timezone offset
   -I < count >
                  -- Number of data to print per line
   -oh < flag >
                  -- Set Output Header Flag (default FALSE)
   -od<flag>
                  - Set Output Data Flag (default TRUE)
                   -- Set Maximum Count Value
   -m<max>
       <flag> can be: 0 (FALSE) or 1 (TRUE).
```

Systems Research Laboratories, Inc. Research & Development Computer Software Delivery Order 0009, Sequence 1, Attachment 2

ACTIGRAF may be run with zero or more options and a single filename. If all of ACTIGRAF's default settings are acceptable to you, **ACTIGRAF** < filename > (where < filename > is the name of a file downloaded by MINI-RDR) will output the contents of the file in a standard ASCII format. This format is machine-readable, but should be acceptable to humans as well.

If you want to save the output from ACTIGRAF, rather than just viewing it on the screen, use the DOS redirection facility. For example,

processes the file 555\_0802.DAT (which was previously produced by MINI-RDR) and place the output into a file named 555\_0802.PRN.

ACTIGRAF outputs header information as well as the activity information. The header information is kept "separate" from the activity information. By default, the header information is output in such a way that it will not be "captured" by DOS redirection. If you need to capture the header information with the activity information, specify the -oh1 option:

If you want header information only, turn on the header information with the -oh1 option, and turn off the activity information with the -od0 option:

The other options may be used similarly depending on your needs.

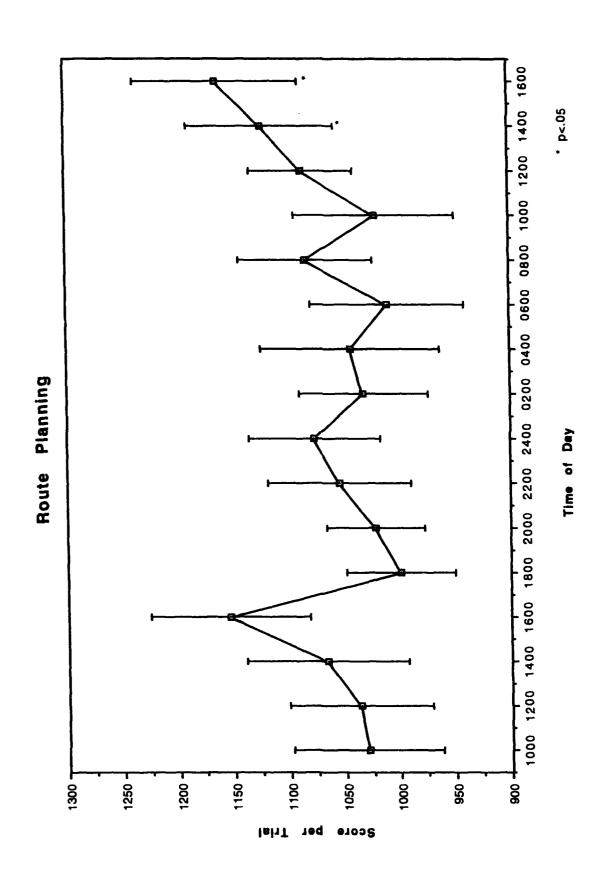
```
actigraf.c
            Name:
*/
#include <stdio.h>
#ifdef VMS
#include <file.h>
#else
#include <fcntl.h>
#endif
#ifdef DOS
#define read _read
#endif
#ifndef FALSE
#define FALSE ( 0)
#define TRUE ( !FALSE)
#endif
#define LABEL_START 0
#define LABEL LEN 18
#define UNK1 START (LABEL START+LABEL LEN)
#define UNK1_LEN 27
#define PACKING_START (UNK1_START+UNK1_LEN)
#define PACKING_LEN 1
#define EPOCH_START (PACKING_START+PACKING_LEN)
#define EPOCH_START (PACKING_START+PACKING_LEN #define EPOCH_LEN 8 #define STOPTIME_START (EPOCH_START+EPOCH_LEN) #define TIME_LEN 8 #define WAKEUP_START (STOPTIME_START+TIME_LEN) #define DATE_LEN 12
#define CURRENT_START (WAKEUP_START+DATE_LEN)
#define ID_START (CURRENT_START+DATE_LEN)
#define ID_LEN 12
#define UNK2_START (ID_START+ID_LEN)
#define UNK2 LEN 30
#define POST_HEADER (UNK2_START+UNK2_LEN)
char filename[81];
char label[LABEL LEN + 1];
char packing[PACKING_LEN + 1];
char epoch[EPOCH_LEN + 1];
char stoptime[TIME_LEN + 1];
char wakeuptime[DATE_LEN + 1];
char currenttime[DATE_LEN + 1];
char id[ID_LEN + 1];
int acti_fd = -1;
int num_dp = 0;
int bits_per_point = 8;
 int start_set = FALSE;
int start_julian;
int start hour;
 int start_minute;
 int current_julian;
 int current hour;
 int current_minute;
 int epoch minutes;
 int epoch_counter;
```

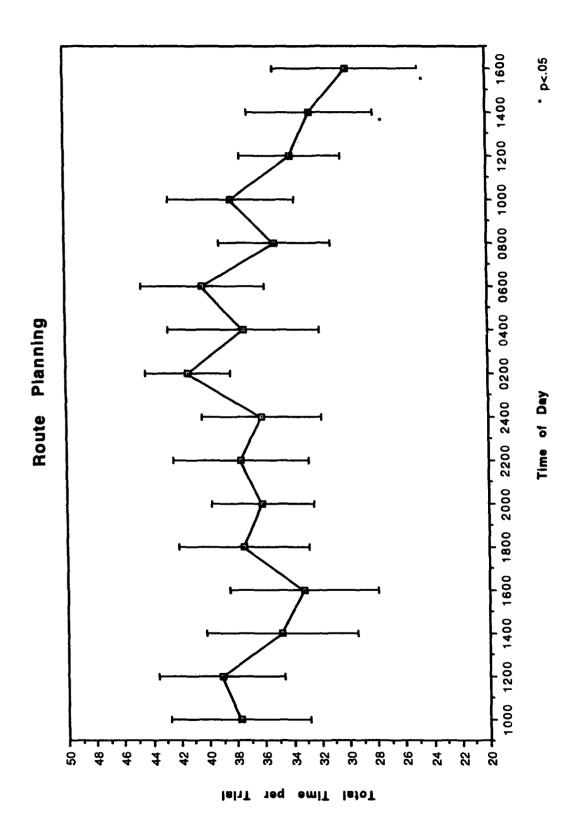
```
unsigned.long epoch_sum;
int bucket_set;
int bucket_size;
int count_on_line;
int max_on_line;
int select_set;
int select_julian_start;
int select_julian_stop;
int argument_errors;
int hour_offset;
int auto_hour_set;
FILE *ofp;
int output_header;
int output_data;
int max_set;
int max_count;
int download_julian;
download_hour;
     download_minute;
int download_second;
```

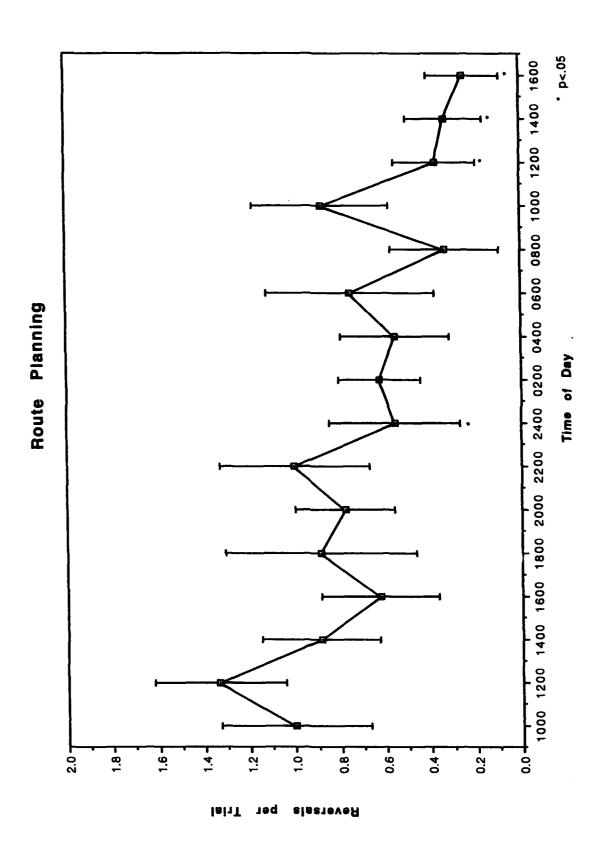
```
main( argc, argv)
int argc:
;har *argv[];
       extern char filename[];
       extern int unpack bits();
        extern int acti_byt();
       extern void acti proc();
        extern int num dp;
        extern int bits per point;
        extern int bucket size;
        extern int bucket set;
        extern int max on line;
        extern int select_set;
        extern int select_julian_start;
        extern int select julian stop;
        extern int argument errors;
        extern int hour offset;
        extern int auto hour set;
        extern FILE *ofp;
        extern int output header;
        extern int output data;
        extern int max set;
        extern int max count;
        int filename cnt;
        int i;
        int i_value;
        char dummy[81];
        char omode;
        FILE *save fp;
        argument errors = 0;
        bucket set = FALSE;
        bucket size = 1;
        hour offset = 0;
        auto_hour_set = FALSE;
        max on line \approx 1;
        select set = FALSE;
        filename cnt = 0;
        ofp = stdout;
        output header = FALSE;
        output data = TRUE;
        max set = FALSE;
         for (i = 1; i < argc; i ++)
                 if( argv[i][0] == '-')
                          if( strncmp( &argv[i][1], "ah", 2) == 0)
                                  auto hour_set = TRUE;
                          else if( strncmp( \&argv[i][1], "b", 1) == 0)
                                  if( sscanf( &argv[i][2], "%d%s",
                                  &i_value, dummy) != 1)
                                          illegal option( argv[i]);
```

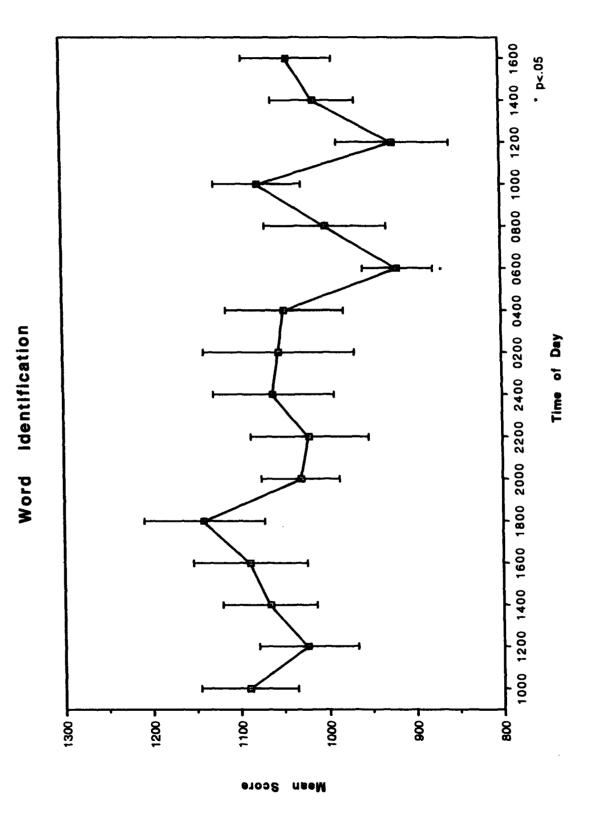
```
continue:
        bucket size = i value;
        bucket set = TRUE;
else if( strncmp( &argv[i][1], "d", 1) == 0)
        if( sscanf( &argv[i][2], "%d%s",
        &i_value, dummy) != 1)
                illegal_option( argv[i]);
                continue;
        select_julian_start = i_value;
        select_julian_stop = i_value;
        select set = TRUE;
else if (strncmp(argv[i][1], "h", 1) == 0)
        if( sscanf( &argv[i][2], "%d%s",
        &i value, dummy) != 1)
                illegal option( argv[i]);
                continue;
        hour offset = i value;
else if (strncmp(&argv[i][1], "l", 1) == 0)
        if( sscanf( &argv[i][2], "%d%s",
        &i value, dummy) != 1)
                 illegal_option( argv[i]);
                 continue;
                 }
        max on line = i value;
else if (strncmp(argv[i][1], "m", 1) == 0)
         if( sscanf( &argv[i][2], "%d%s",
         &i value, dummy) != 1)
                 illegal option( argv[i]);
                 continue:
                 }
         max count = i_value;
         max set = TRUE;
else if( strncmp( &argv[i][1], "o", 1) == 0)
         omode = argv[i][2];
         if ( omode != 'h' && omode != 'd')
                 illegal_option( argv[i]);
                 continue;
         if( sscanf( &argv[i][3], "%d%s",
         &i value, dummy) != 1)
```

Appendix 8. Performance data from the tests involved in the second fatigue study.









1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 Identification Word 35 49 36 38 37. . 98 20 **48** 46 <del>6</del> 47

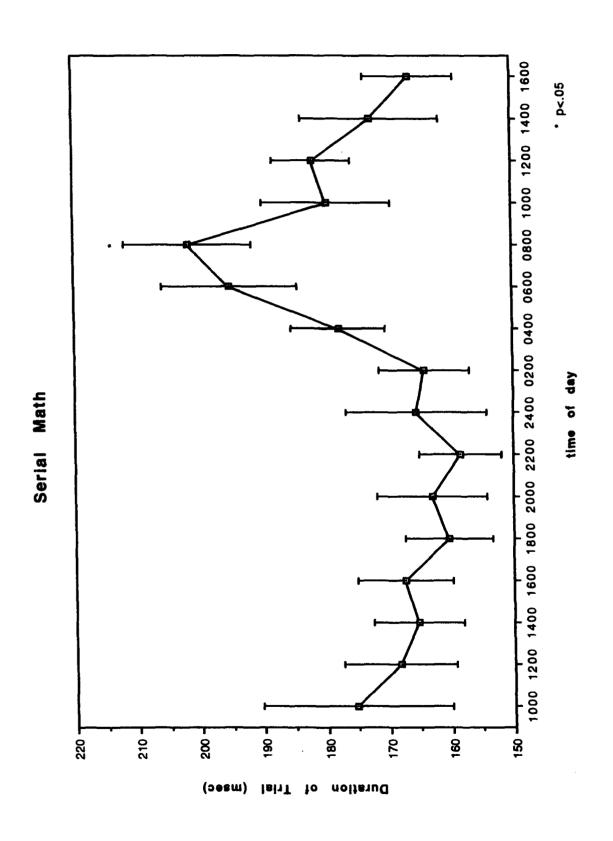
Mean Task Duration

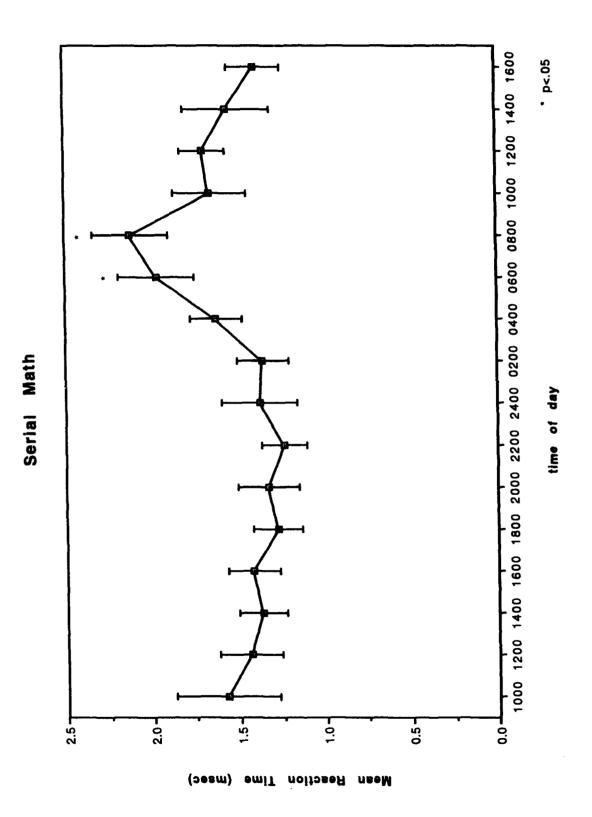
Time of Day

1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 • p<.05 Stanford Sleepiness Survey Time of Day 9 <u>ი</u> 5 2

Mean Score

1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 . p<.05 Survey Sleepiness Time of Day Stanford 4 16 -12 -<del>1</del> 0 + 18 1 ė 8 Trial Duration (msec)

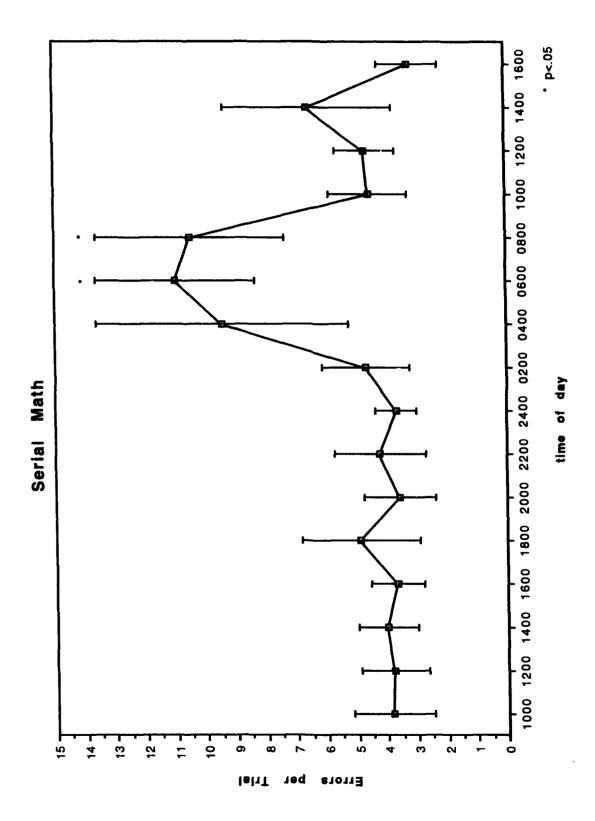


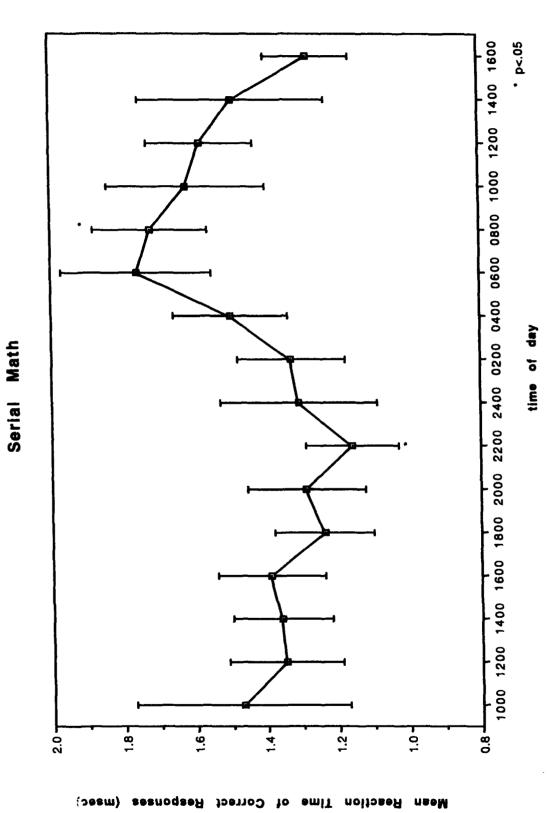


Minimum Reaction Time (msec)

Serial Math

time of day





1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 Wilkinson's Four Choice Reaction Time 8 8 6 8 8 8 8 8 8 8 8 8 8 6 42

Task Duration (maec)

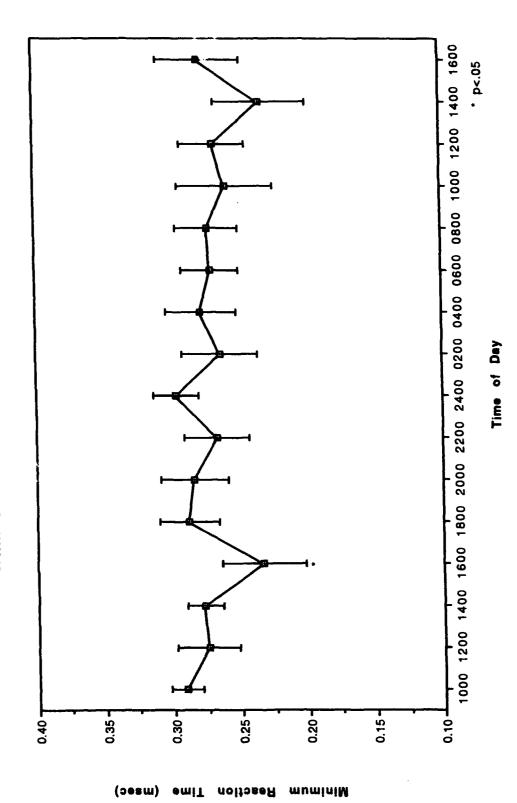
Time of Day

. p<.05

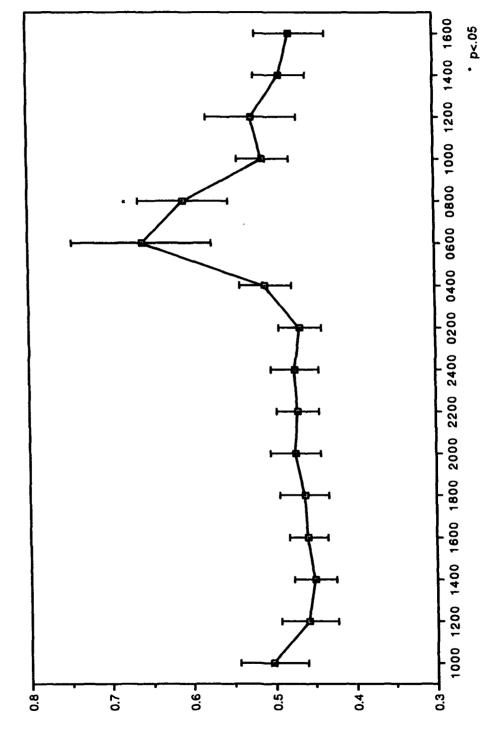
1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 . p<.05 Wilkinson Four Choice Reaction Time (msec) 2.5 2.0-1.5 0.0 4.5-3.5 3.0-0.1 0.5 5.0 4.0 emiT Maximum Reaction

Time of Day

Wilkinson's Four Choice Reaction Time



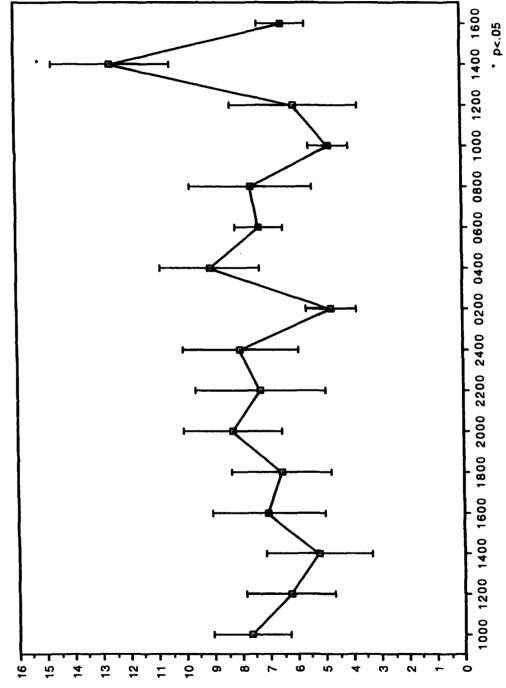
Wilkinson's Four Choice Reaction Time



Correct Responses (msec)

Time of Day

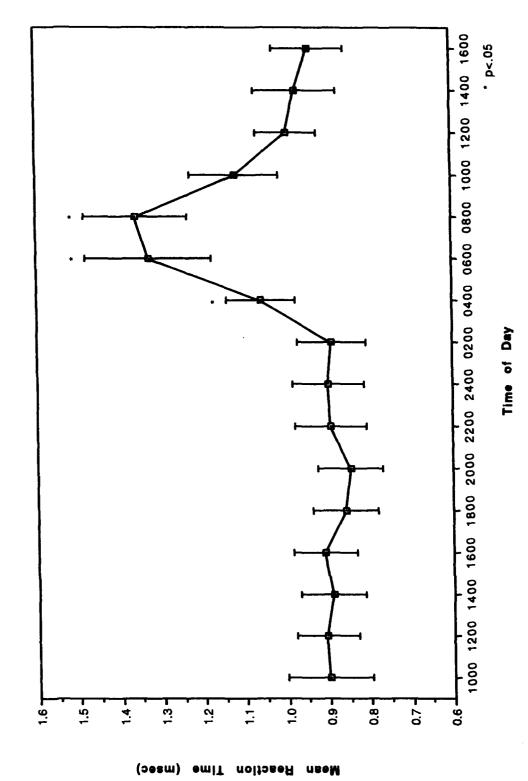
**Numbers and Words** 



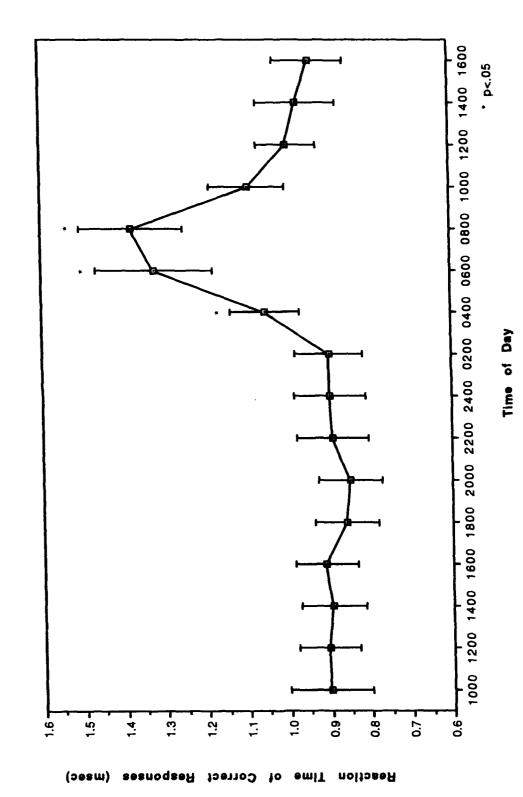
Time for incorrect"Hits" (msec)

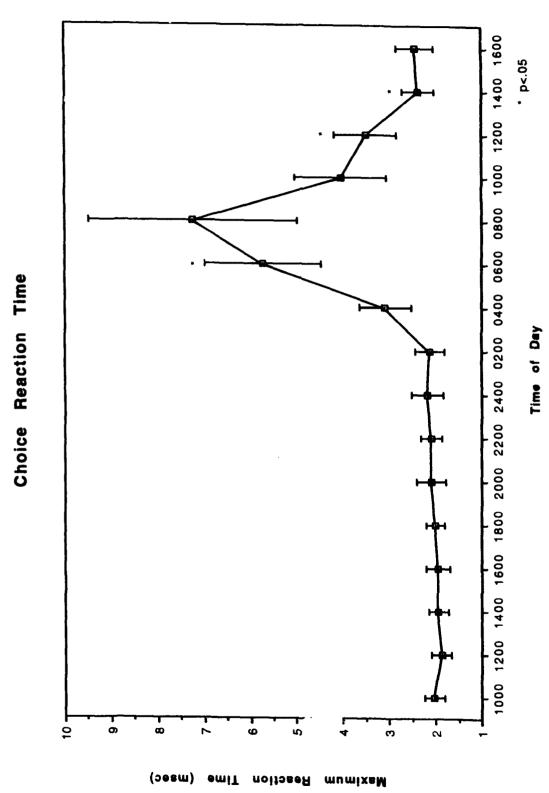
Time of Day

Choice Reaction Time



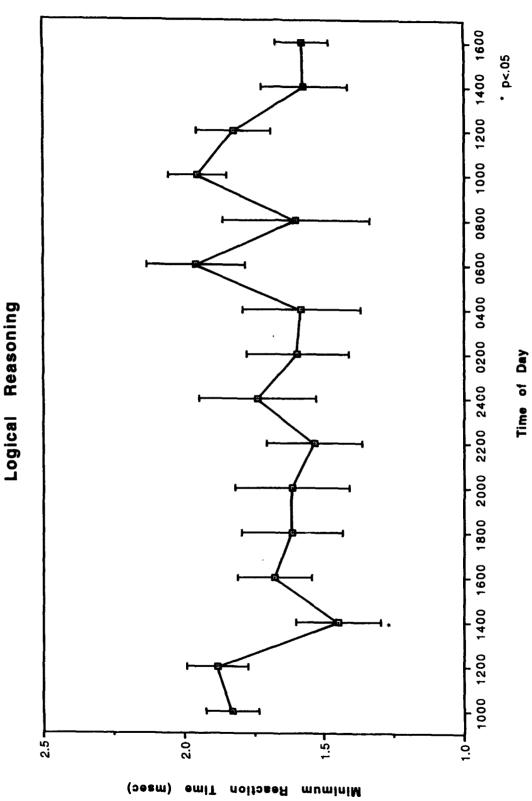
Choice Reaction Time

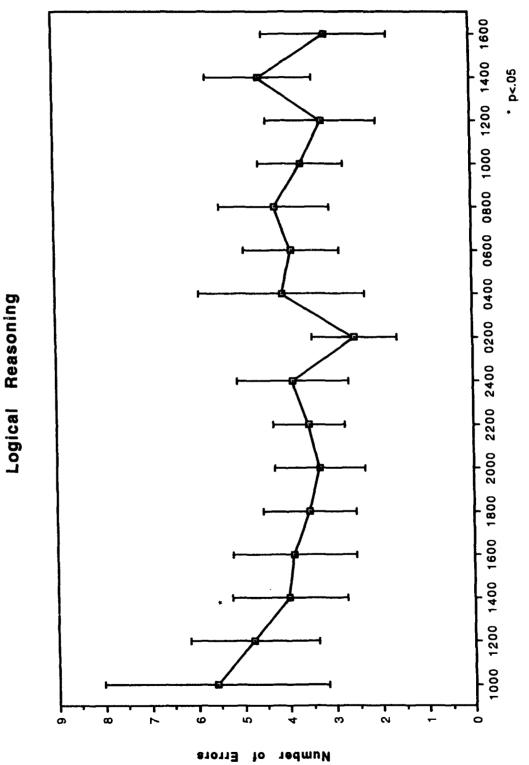




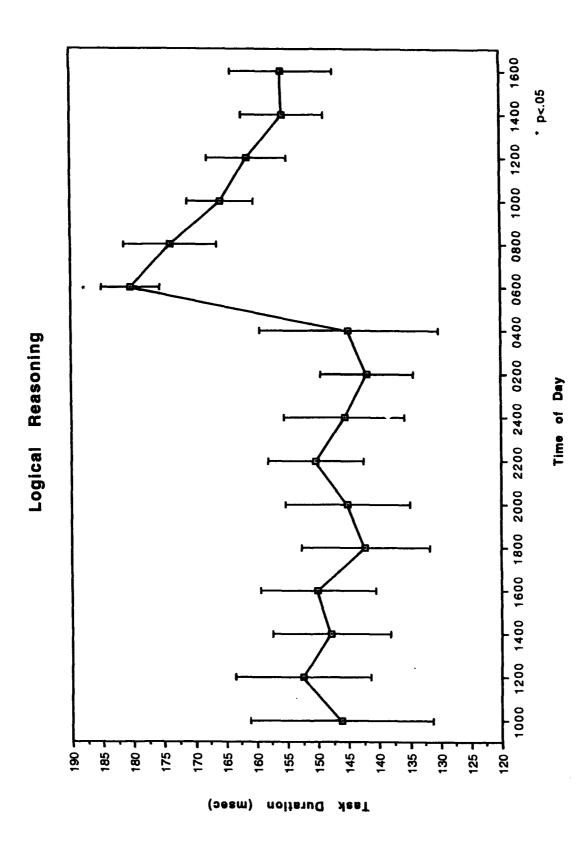
1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200 1400 1600 . p<.05 Logical Reasoning **7**22 38 8 . Ж 8 29 **5**8 27. **5**6. ဓ္ထ ည Rigit to redmul

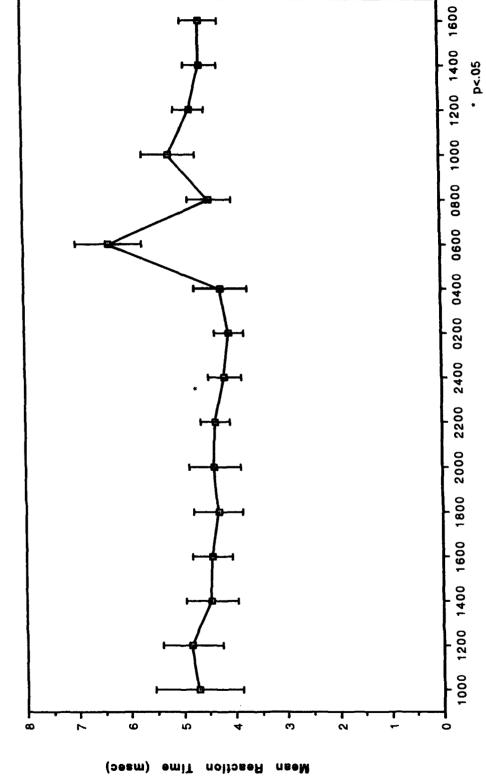
Time of Day





Time of Day

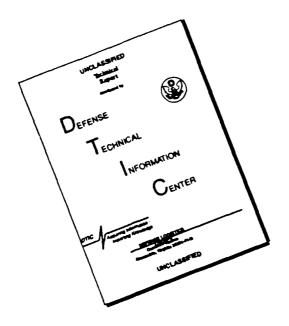




Logical Reasoning

Time of Day

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